

A COMPARATIVE STUDY OF THE RATE OF METABOLISM
OF CERTAIN AMINO ACIDS.

BY MARGARET WOODWELL JOHNSTON.

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN THE
UNIVERSITY OF MICHIGAN

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COMPARATIVE STUDIES OF THE METABOLISM OF AMINO ACIDS.

I. CHANGES IN THE NON-PROTEIN NITROGENOUS CONSTITUENTS OF THE BLOOD FOLLOWING ADMINISTRATION OF AMINO ACIDS.*

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Since the discovery of the presence of amino acids in the blood by Delaunay (1) and Van Slyke and Meyer (2) and the subsequent confirmation by Abderhalden (3) and Abel, Rowntree, and Turner (4), attention of students of metabolism has been focused on the behavior of the individual amino acids, components of the protein molecule, in the solutions of the problems which involve the behavior of protein in the organism. Earlier studies, for the most part, were confined to investigations of the changes in the composition of the urine after amino acid administration. Relatively few reports are available in which blood studies by the newer methods of blood analysis were carried out, and, for the most part, these are limited to a few amino acids. In this paper, we present the results of blood analyses after the administration enterally and subcutaneously of glycine, *dl*-alanine, *d*-alanine, *dl*-aspartic acid, *d*-glutamic acid, *d*-lysine, and *d*-arginine. In many cases, a number of these acids have been fed to the same animal, so that to some extent, the factor of individual variability is ruled out. The literature which bears upon our experiments will be discussed later in connection with the consideration of our experimental results.

* The material presented here represents an abstract of part of the thesis presented by Margaret Woodwell Johnston in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the University of Michigan.

EXPERIMENTAL.

The experimental animals were male rabbits maintained upon a standard weighed diet of oats and either carrots or cabbage,¹ which was fed at the end of the 6 or 12 hour experimental period, the time of feeding being always the same throughout any individual experiment. In order to insure an adequate fluid intake, 100 cc. of water were given daily by tube in addition to 100 cc. placed in the cage. On control days, the water was administered at the beginning of the first period of the day. On experimental days half the water was fed at the time of the administration of the amino acid and the remainder was administered 3 hours later.

The amino acids used were glycine, *dl*-alanine, *d*-alanine, *d*-glutamic acid, *dl*-aspartic acid, *d*-lysine, and *d*-arginine. Of these, the glutamic acid and lysine were prepared in this laboratory. In addition, a mixture of amino acids (aminoids from milk) was fed. The amount administered in every case, unless otherwise noted, was equivalent to 0.182 gm. of amino nitrogen per kilo of body weight. For the oral administration, glycine, alanine, and arginine were dissolved in 50 cc. of water, while glutamic and aspartic acids were given as the sodium salts in the same volume of fluid. Lysine dihydrochloride was treated with the theoretical amount of sodium hydroxide to neutralize the hydrochloric acid before administration. In those experiments in which the amino acids were given subcutaneously, each acid was dissolved in as small a volume of water as possible and injected. In order to maintain a constant fluid intake, additional water sufficient to give a total volume of 50 cc. as in the feeding experiments, was introduced by stomach tube.

The animals were placed on the weighed diet several days before the beginning of the experiment. In those experiments in which the same rabbit was used as subject repeatedly, a period of at least 2 weeks, usually more, elapsed between successive experimental periods. On the experimental days, blood was drawn from the marginal ear vein before the administration of the amino acid and again at 3, 6, 12, and usually 30 hours following the adminis-

¹ In a few early experiments the rabbits were fed a diet of milk and cane sugar. This, however, was later changed to the carrot-oat diet.

tration. Potassium oxalate was used as an anticoagulant. The bloods were immediately deproteinized and filtered, and determinations of the distribution of non-protein nitrogen were made by the methods of Folin. The term "undetermined" nitrogen in the tables is used to designate non-protein nitrogen, not urea or amino nitrogen.

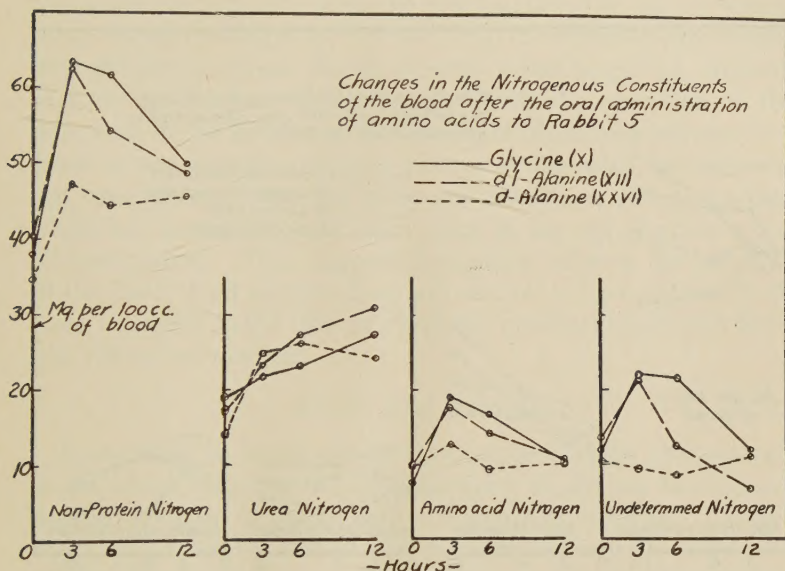


CHART I. The changes in the distribution of non-protein nitrogen of the blood of Rabbit 5, following the oral administration of glycine, *dl*-alanine, and *d*-alanine, equivalent to 0.182 gm. of nitrogen per kilo.

RESULTS.

A. Feeding Experiments.

The results of the experiments with Rabbit 5, in which the entire series of amino acids studied was fed to the same animal, are presented graphically in Charts I and II, in which are shown the variations in the amounts of the nitrogenous constituents of the blood and the time relationships observed. These curves are fairly representative of the typical changes observed in other

feeding experiments as will be evident by a comparison of these results with those of Table I.²

Urea Nitrogen.

The maximal increase in the urea nitrogen of the blood at the end of the 3 hour period after oral administration of glycine was

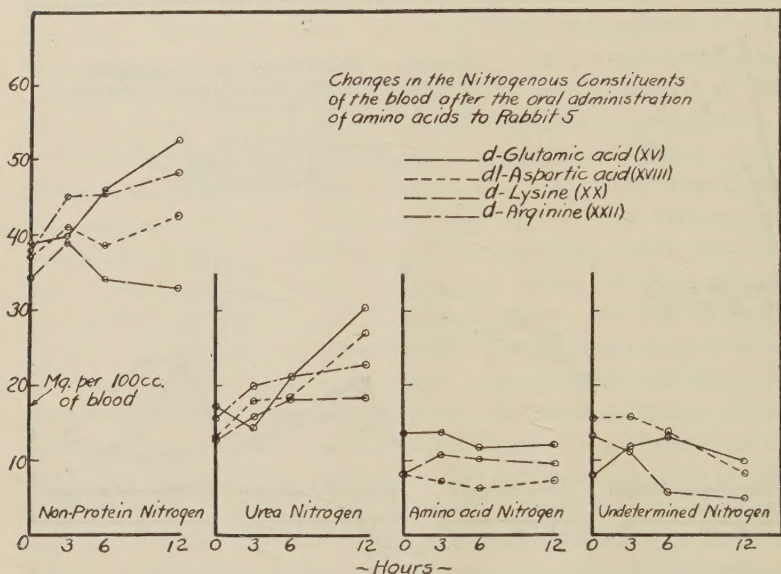


CHART II. The changes in the distribution of non-protein nitrogen of the blood of Rabbit 5, following the oral administration of *d*-glutamic acid, *dl*-aspartic acid, *d*-lysine, and *d*-arginine, equivalent to 0.182 gm. of nitrogen per kilo.

3.6 mg. per 100 cc., an increase entirely comparable with those increases obtained at a similar period after the ingestion of equiva-

² With glycine, *dl*-alanine, and *d*-glutamic acid, a very considerable number of experiments, of which those presented are typical, were carried out (seventeen with glycine and nine each with *dl*-alanine and glutamic acid). In the columns headed "Experiment No." of the tables, the arabic numerals indicate the laboratory number of the animals used. It is thus possible to compare results with the same animal after the feeding or injection of different amino acids.

lent amounts of amino nitrogen as arginine and lysine. When, however, *dl*-alanine was fed, the maximal increase in urea nitrogen at this time was greater, 7.4 mg. The maximal increases in urea nitrogen following similar ingestion of *d*-alanine, *d*-glutamic acid, and *dl*-aspartic acid were 10.6 mg., 10.4 mg., and 4.9 mg. respectively. An increase of blood urea nitrogen of 10.4 mg. at the end of 3 hours, after glutamic acid had been fed, did not always occur (compare Chart I), but in a total of seventeen experiments with glycine, in no case was an increase of this magnitude observed as early as 3 hours after feeding. With the exception of this difference in the initial rate of increase in blood urea nitrogen as a result of the ingestion of these various amino acids, the course of the urea nitrogen appears quite comparable, a steady increase, which has reached its maximum value at the end of either a 6 or 12 hour period. The long period elapsing between the collection of the later blood samples does not warrant a more definite statement in regard to the time at which the maximal blood urea nitrogen values are reached.

Amino Acid Nitrogen.

In contrast to the relatively slow increase in the urea nitrogen of the blood following the administration of glycine, an early and marked increase in amino acid nitrogen was observed. In most cases, the value for amino acid nitrogen obtained 3 hours after feeding represented the maximal value observed for this constituent of the blood. It is interesting to note, however, that the amino acid nitrogen of the blood had not returned to its normal fasting level 12 hours after the feeding of glycine. The changes in the amino acid nitrogen of the blood, after *dl*-alanine had been fed, were similar to those obtained after the feeding of glycine, except that usually the maximal value obtained was somewhat lower. When, however, *d*-alanine was fed, either no increase in amino acid nitrogen was observed or if an increase was observed it was slight and transitory.

With one exception, the administration of *d*-glutamic and *dl*-aspartic acid failed to influence the amino acid nitrogen of the blood. In the one exceptional case the maximal increase observed was only 3.1 mg., a slight effect when contrasted with the rises of 10 to 20 mg. observed after the feeding of glycine.

TABLE I.

Changes in the Distribution of Nitrogenous Constituents of Blood Following the Oral Administration of Amino Acids.

All results are expressed in mg. per 100 cc. of whole blood. The amount of amino acid administered was equivalent to 0.182 gm. of nitrogen per kilo of body weight.

Experiment No.	Duration.	Non-protein N.	Urea N.	Amino acid N.	Undetermined N.
	<i>hrs.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
III-1. Glycine.	0	54.3	22.1	12.6	19.6
	3	85.8	23.0	21.5	41.3
	6	75.2	22.7	22.9	29.6
	12	66.3	28.7	20.0	17.6
	30	57.8	25.0	15.3	17.5
V-3. Glycine.	0	40.8	15.6	9.2	16.0
	3	76.0	17.9	22.8	35.3
	6	73.4	20.4	23.8	29.2
	12	55.8	19.0	20.7	16.1
	30	40.0	18.1	9.9	12.0
VI-3. Glycine.	0	42.8	19.4	10.1	13.3
	3	74.6	19.9	22.6	32.1
	6	71.4	21.1	19.9	30.4
	12	64.4	23.0	18.5	22.9
	30	50.0	22.6	12.7	14.7
VIII-4. Glycine.	0	41.7	20.0	8.8	12.9
	3	67.8	23.6	22.2	22.0
	6	67.0	29.1	17.8	20.1
	12	56.2	28.6	13.1	14.5
	30	39.7	21.4	9.4	8.9
IV-1. <i>dl</i> -Alanine.	0	50.0	22.7	13.5	13.8
	3	73.2	30.1	18.4	24.7
	6	68.8	32.4	19.1	17.3
	12	70.3	37.5	14.5	18.3
	30	57.9	30.0	9.6	18.3
XXVIII-8. <i>dl</i> -Alanine.	0	40.8	21.8	8.2	10.8
	3	59.0	27.9	17.9	13.2
	6	53.6	28.3	14.7	10.6
	12	53.0	27.5	11.2	14.3
	30	40.9	22.7	8.3	9.9
XXV-8. <i>d</i> -Glutamic acid.	0	39.8	22.8	8.1	9.0
	3	49.8	33.2	8.9	7.7
	6	44.7	32.1	7.2	5.4
	12	46.8	30.4	7.3	9.1
	30	40.0	29.2	7.4	3.4

TABLE I—*Concluded.*

Experiment No.	Duration.	Non-protein N.	Urea N.	Amino acid N.	Undetermined N.
	<i>hrs.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
XIII-4. <i>d</i> -Glutamic acid.	0	33.6	14.8	7.1	11.7
	3	40.2	17.1	10.3	12.8
	6	41.0	21.1	10.0	9.9
	12	42.7	24.4	6.6	11.7
	30	33.3	14.9	5.9	12.4
XXI-8. <i>d</i> -Lysine.	0	37.0	18.1	8.1	10.8
	3	39.8	21.1	11.3	7.4
	6	41.0	20.5	10.7	9.8
	12	42.5	26.7	9.2	6.6
	30	36.0	20.4	8.1	7.5
XXXI-11. Glycine.*	0	38.2	16.5	9.6	12.1
	3	52.1	19.5	17.1	15.5
	6	78.1	21.3	23.8	25.0
	12	59.2	33.5	9.8	15.9
	30	39.1	17.8	8.6	12.7

* Glycine equivalent to 0.182 gm. of nitrogen per kilo was ingested at the usual time and an equal dose 3 hours after the first administration.

The oral administration of lysine (two experiments only) did not markedly affect the amino acid nitrogen of the blood. Our experiments with arginine do not afford much information, since it is known that arginine does not react quantitatively with the reagent employed by Folin. Although no values for the amino acid nitrogen of the blood could be obtained, the changes in the non-protein nitrogen not urea nitrogen of the blood would lead us to believe that the feeding of arginine had little influence on the amino acid nitrogen of the blood.

Non-Protein Nitrogen and Undetermined Nitrogen.

The changes in the non-protein nitrogen of the blood following the feeding of amino acids, except glycine and *dl*-alanine, were such that they could be adequately explained by the simultaneous increases in the urea nitrogen. When, however, glycine was fed at the end of the 3 hour experimental period, a marked increase in non-protein nitrogen was observed which could not be explained satisfactorily by the increases in amino acid and urea nitrogen

observed at the same time. In other words, an increase in undetermined nitrogen of the blood was observed, which in the whole series of seventeen experiments varied from 4.5 to 21.1 mg. per 100 cc. In two of the seven feeding experiments with *dl*-alanine, a definite increase in this undetermined nitrogen also occurred. The maximal increase in the non-protein nitrogen of the blood after the

TABLE II.

Changes in the Distribution of Nitrogenous Constituents of Blood Following the Administration of Mixtures of Amino Acids (Aminoids).

All results are expressed in mg. per 100 cc. of whole blood. The amount of amino acid administered was equivalent to 0.182 gm. of nitrogen per kilo of body weight.

Experiment No.	Duration.	Non-protein N.	Urea N.	Amino acid N.	Undetermined N.
	<i>hrs.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
XXXV-11. Aminoids.	0	48.9	20.2	12.1	16.6
	3	55.4	26.4	14.0	15.0
	6	49.8	22.9	10.7	16.2
	12	28.3	23.2	8.9	16.2
XLIV-11. Aminoids.*	0	51.6	22.7	11.7	17.2
	3	60.1	28.0	15.1	17.0
	6	52.0	29.0	11.8	11.2
	12	53.0	30.1	9.6	13.3
	30	41.8	18.6	7.7	15.5
XXXVI-11. Aminoids and glycine.†	0	45.1	20.1	12.1	12.9
	3	71.2	25.5	24.4	21.3
	6	69.0	26.9	20.3	21.8
	12	55.8	28.8	11.9	15.1
	30	47.3	23.3	10.5	13.5

* The amount of nitrogen fed was twice the usual amount.

† The amount of nitrogen fed was twice the usual amount; one-half as aminoid nitrogen and one-half as glycine nitrogen.

ingestion of glycine or *dl*-alanine appeared to occur simultaneously with the maximal amino acid nitrogen value. In the case of the other amino acids studied, the appearance of the maximal rise in non-protein nitrogen was determined by the height of the urea nitrogen fraction.

In the hope of accentuating these striking increases in the undetermined nitrogen, observed after the feeding of glycine, a second

feeding of the usual amount of glycine was given 3 hours after the first. The second administration of glycine further increased the

TABLE III.

Changes in the Distribution of Nitrogenous Constituents of Blood Following the Subcutaneous Injection of Amino Acids.

All results are expressed in mg. per 100 cc. of whole blood. The amount of amino acid administered was equivalent to 0.182 gm. of nitrogen per kilo of body weight.

Experiment No.	Duration.	Non-protein N.	Urea N.	Amino acid N.	Undetermined N.
	<i>hrs.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
XXXIV-11. <i>d</i> -Glutamic acid.	0	39.3	20.1	11.2	8.0
	3	63.7	26.7	23.6	13.4
	6	64.7	33.4	16.2	15.1
	12	65.3	44.0	9.4	11.9
	30	49.2	33.2	7.8	8.2
XL-16. <i>d</i> -Glutamic acid.	0	36.4	15.8	9.7	10.9
	3	65.6	24.0	30.8	10.8
	6	65.6	38.5	14.0	13.1
	12	73.2	57.4	6.4	9.4
	30	50.5	29.8	7.5	13.2
XXXIII-11. Glycine.	0	36.1	18.3	8.3	9.5
	3	58.0	23.6	19.8	14.6
	6	55.7	26.4	14.4	14.9
	12	49.1	28.7	9.6	10.8
	30	43.2	27.3	9.0	6.9
XXXVIII-15. <i>dl</i> -Alanine.	0	51.5	21.0	11.4	19.1
	3	59.2	23.0	18.6	17.6
	6	52.2	25.6	13.6	13.0
	12	67.3	26.5	12.1	28.7
	30	43.7	21.1	7.5	15.1
XLV-16. <i>d</i> -Alanine.	0	48.5	18.5	9.8	20.2
	3	56.5	28.3	10.3	18.0
	6	47.0	29.2	8.1	9.7
	12	40.2	24.6	6.6	9.0
	30	33.8	15.7	9.0	9.1

undetermined nitrogen significantly in one experiment (Table I) and not in a second. Oral administration of a mixture of amino acids containing an amount of nitrogen equivalent to that usually

fed (aminoids from milk) failed to produce a significant increase in undetermined nitrogen (Table II). When twice the usual amount of nitrogen was fed as aminoids, essentially the same results were obtained. In this connection, it should be pointed out that it is usually stated that the proteins of milk contain no glycine and hence, if glycine is the chief amino acid contributing to the rise in the undetermined nitrogen of the blood, negative results such as we obtained might be anticipated. In further support of this point of view, are results obtained when aminoids and glycine were fed together (Table II), which showed increases in the non-protein nitrogen, amino acid nitrogen, and undetermined nitrogen, similar to those observed when glycine was fed alone.

B. Subcutaneous Administration.

Glycine, *dl*-alanine, *d*-alanine, and *d*-glutamic acid were also injected subcutaneously. With the exception of *d*-glutamic acid this mode of administration produced essentially the same changes as observed in the feeding experiments (Table III). The subcutaneous administration of *d*-glutamic acid was followed by a marked increase in the amino acid nitrogen of the blood obtained 3 hours after the amino acid was injected. This was also accompanied by a marked increase in the non-protein nitrogen and in the undetermined nitrogen.

DISCUSSION.

The results obtained suggest two factors which may possibly influence the rate of metabolism of the amino acids, the rate of absorption from the gastrointestinal tract, and the rate of deamination and subsequent formation of urea. The early and marked increases in non-protein nitrogen and amino acid nitrogen, after glycine and *dl*-alanine were fed, indicate a rapid absorption at a rate not widely different for the two amino acids. Cori (5), in his studies on rats, found that the rates of absorption of glycine and *dl*-alanine from the gastrointestinal tract were 0.048 and 0.045 gm. respectively per 100 gm. of body weight. While it is realized that caution must be exercised in interpreting figures obtained for rats as significant for other species, if these findings

hold approximately for the rabbit, the amounts of glycine and *dl*-alanine used in our experiments should be effectively absorbed within 3 hours, the period at the end of which our first blood samples were taken. The similarity of the results obtained when these two amino acids were administered orally and subcutaneously is additional evidence for the belief that absorption from the gastrointestinal canal in the feeding experiments must have been rapid.

When, however, the dicarboxylic acids, *d*-glutamic and *dl*-aspartic acid, were fed, no significant increase in the amino acid content of the blood was observed and a slow increase in non-protein nitrogen occurred which was accompanied by a proportionate rise in urea nitrogen. That this difference in the curves of non-protein nitrogenous constituents of blood may be due, in part at least, to a slower rate of absorption of the dicarboxylic amino acids from the alimentary canal has been suggested by Seth and Luck (6). Further evidence in support of this view-point is obtained from a consideration of our results following the subcutaneous injection of glutamic acid. In these experiments, the early increases in the non-protein, amino acid, and undetermined nitrogen were entirely comparable to those observed after glycine was administered. It should be noted, however, that both the urea and non-protein nitrogen reached very high levels, which suggested a possible toxic effect with some retention of urea. Complete urine analyses were made in all experiments and in Experiment XL (Table III), only 7 cc. of urine were excreted in the 12 hours immediately following the injection of the glutamic acids, while in Experiment XI (not presented in the tables), a marked although not so striking a decrease in the output of urine occurred. Some evidence of the toxicity of glutamic acid is presented in the literature. Stolte (7) reported the death of a rabbit following the intravenous injection of glutamic acid. Lewis, Dunn, and Doisy (8) have observed some toxic effects accompanied by an oliguria in man after feeding 20 gm. of glutamic acid. In the present series, one animal died within 6 hours after the ingestion of two doses of glutamic acid, administered 3 hours apart. For these reasons, we hesitate to interpret the very high urea nitrogen values obtained in the later periods after injection of glutamic acid as significant and indicative of the extent of deamination of this amino acid.

The only other amino acid which was administered both orally and subcutaneously, *d*-alanine, appeared to be absorbed as readily as were glycine and *dl*-alanine.

If it is assumed that the rate of absorption of glycine and *dl*-alanine are essentially the same, some other factor must be responsible for the fact that, as a rule, the amino acid nitrogen of the blood failed to reach as high a level after alanine was fed as after glycine. This, together with the fact that the rate of increase of the blood urea nitrogen was greater when *dl*-alanine was fed, is suggestive of a difference in the rate of deamination of the two amino acids. The suggestion that, although the rate of absorption from the alimentary canal may be essentially the same for alanine and glycine, the metabolism of glycine may proceed more slowly than that of *dl*-alanine, is further supported by the fact that analyses of the urine fractions, corresponding in periods of time to the collection of blood samples, frequently indicated a depression or at least no increase in urea excretion in the 6 hours immediately following the administration of glycine.

If *d*-alanine, on the other hand, is absorbed as rapidly as are glycine and *dl*-alanine, its rate of deamination must be greater than that of either of these two amino acids, since in all experiments with *d*-alanine, there occurred an early increase in urea nitrogen and no significant increase in amino nitrogen of the blood. It is possible that the differences in the behavior of *dl*-alanine and *d*-alanine may be related to the fact that the latter is the optically active form of the amino acid, which occurs in the living organism.

Our data are also of interest as far as concerns the time relationships of the maximal values for amino acid and urea nitrogen. Whenever increases of amino nitrogen of the blood were observed, these always preceded the maximal increase of urea nitrogen of the blood, regardless of whether the amino acids were administered *per os* or subcutaneously. This is in agreement with the results of Folin and Berglund (9) in man, of Seth and Luck (6) in dogs and rabbits, and of Lewis and Izume (10) in rabbits, but contrary to the results of Van Slyke (11) and of Morgulis (12) in dogs. Both of these investigators, who, however, fed proteins rather than amino acids, observed that the increase in urea nitrogen may precede the increase in amino acid nitrogen. It is well to consider carefully the suggestion of Fearon (13) that the dog

may differ from other animals in that the processes of deamination and urea synthesis are not histologically separated to the same extent, but may be chiefly confined to one organ.

In an attempt to determine the origin and significance of the rises in undetermined nitrogen observed in our experiments with glycine, we have attempted to apply the method suggested by Swanson (14) for the determination of peptide nitrogen in Folin-Wu blood filtrates. The method proved to be so unsatisfactory for the small amounts of blood which were available in our experiments that our failure to observe significant changes in peptide nitrogen cannot be considered as excluding the presence of this type of nitrogenous compound in the blood.

Swanson (14) considered that the peptide nitrogen of human blood accounted for the greater part of the undetermined (rest) nitrogen, while Blau (15) was unable to demonstrate significant amounts of peptide nitrogen in normal human blood. Kalmykoff (16) was unable to determine the presence of peptide nitrogen in dialysates of fasting blood from different blood vessels of a dog, but as absorption of protein digestion products from the gastrointestinal canal began, polypeptide nitrogen appeared in the blood. He observed also that this polypeptide nitrogen of the portal blood was less than that of the hepatic blood, while in the case of the amino nitrogen of the bloods, the conditions were reversed. He suggested a synthesis of polypeptides from amino acid in the liver. Kotschneff (17), in a continuation of this work, after the introduction of various amino acids (*dl*-leucine, *dl*-valine, "rectamin") into the duodenum of the dog, observed that the normal value of peptide nitrogen (3 to 4 mg. per 100 cc. of blood) was increased to 8.1 to 9.9 mg. in femoral blood, with values of 11.4 to 12.5 mg. in portal blood. This would indicate synthesis of peptides from amino acids in the organism of the dog. Kotschneff considers that the synthesis may take place, not only in the liver, but also in the kidney and muscle as well, since more polypeptide nitrogen was found in the blood from the renal and femoral veins than in arterial blood, after the injection of amino acids into the jugular vein. On the other hand, Van Slyke (11) could find no evidence of an increase in peptide nitrogen in any of the tissues examined following the intravenous introduction of protein hydrolysates to dogs.

Folin and Berglund (9) and Wu (18) have shown that the greater part of the undetermined nitrogen of the blood is contained in the corpuscles. Wu (18) suggests that until further evidence is obtained, the unknown fraction of the nitrogen may be considered as peptide and peptone nitrogen. We consider that the results of these investigators are of significance in relation to our own results since the methods of deproteinization and amino acid determination used by them were the same as those used in the present investigation. Since the various protein precipitants are known to differ in respect to the amount of non-protein nitrogenous material remaining in the filtrate (19), uniform results for the undetermined nitrogen of the blood might not be obtained if different protein precipitants were employed. Abel, Pincoffs, and Rouiller (20) earlier observed that albumoses could be isolated from all the tissues of the body including the formed elements of the blood. They also isolated larger amounts of albumoses from the mucosa of the gastrointestinal tract after a protein meal than in fasting, but did not believe that these substances could be traced from the gastrointestinal tract to the other organs of the body, unless the cellular elements of the blood could be considered as the distributing agents, a conclusion which they felt was not justifiable at that time.

The persistent increases in undetermined nitrogen which we observed after the administration of glycine suggest the possibility of the occurrence of peptide nitrogen in increased amounts in the blood. Why peptide synthesis should occur from glycine and not from the other amino acids studied is not evident. It should be noted, however, that high values for undetermined nitrogen were always associated with a high level of amino acid nitrogen, and that this amino acid nitrogen rose to higher levels following the administration of glycine than of the other amino acids with the exception of glutamic acid subcutaneously injected. In this experiment a high value for the undetermined nitrogen was also observed.

It should also be pointed out that the values for undetermined nitrogen are obtained by difference, and that they are therefore subject to the errors inherent in the other methods of determination. Moreover, little data are available in regard to the manner in which the Folin amino acid reagent reacts with peptides. In

unpublished observations by R. H. Wilson of this laboratory, it was determined that the peptide, diglycyl-cystine, reacted with the Folin reagent to give a value for free amino nitrogen of approximately 50 per cent of the theoretical value. If other peptides react thus in part with their free amino groups in Folin's method, then the values reported by others as well as ourselves for amino acid nitrogen of the blood must be high, including a part of the free amino nitrogen of the peptide. This suggests that our undetermined nitrogen values may be too low and that a part, at least, of the peptide nitrogen, if such exists in blood, is included in the amino acid nitrogen fraction. The parallelism between our high values for amino acid nitrogen and the undetermined nitrogen is at least suggestive and worthy of further investigation. Further work along these lines is in progress.

SUMMARY.

1. The changes in the non-protein nitrogen, urea nitrogen, amino acid nitrogen, and undetermined nitrogen of the blood of the rabbit after the oral and subcutaneous administration of a number of amino acids have been studied.

2. The results suggest that two factors, at least, may influence the rate of metabolism of ingested amino acids, the rate of absorption from the intestine and the rate of deamination in the organism proper. It seems probable that in the rabbit, under our experimental conditions, alanine and glycine are absorbed readily and more rapidly than glutamic acid, but that the deamination of glycine may proceed more slowly than the deamination of the other amino acids studied.

3. A significant increase in the "undetermined" nitrogen of the blood was observed regularly after the administration of glycine and occasionally after *dl*-alanine. The possibility that this may be due to a synthesis of peptide from the amino acid is considered.

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